



# Longevity and temperature response of pollen as affected by elevated growth temperature and carbon dioxide in peanut and grain sorghum

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## ARTICLE INFO

### Article history:

Received 26 February 2010

Accepted 10 August 2010

### Keywords:

Carbon dioxide  
Pollen longevity  
Pollen germination  
Temperature  
Cardinal temperatures

## ABSTRACT

It is important to understand the effects of environmental conditions during plant growth on longevity and temperature response of pollen. Objectives of this study were to determine the influence of growth temperature and/or carbon dioxide (CO<sub>2</sub>) concentration on pollen longevity and temperature response of peanut and grain sorghum pollen. Plants were grown at daytime maximum/nighttime minimum temperatures of 32/22, 36/26, 40/30 and 44/34 °C at ambient (350 μmol mol<sup>-1</sup>) and at elevated (700 μmol mol<sup>-1</sup>) CO<sub>2</sub> from emergence to maturity. At flowering, pollen longevity was estimated by measuring *in vitro* pollen germination at different time intervals after anther dehiscence. Temperature response of pollen was measured by germinating pollen on artificial growth medium at temperatures ranging from 12 to 48 °C in incubators at 4 °C intervals. Elevated growth temperature decreased pollen germination percentage in both crop species. Sorghum pollen had shorter longevity than peanut pollen. There was no influence of CO<sub>2</sub> on pollen longevity. Pollen longevity of sorghum at 36/26 °C was about 2 h shorter than at 32/22 °C. There was no effect of growth temperature or CO<sub>2</sub> on cardinal temperatures (*T*<sub>min</sub>, *T*<sub>opt</sub>, and *T*<sub>max</sub>) of pollen in both crop species. The *T*<sub>min</sub>, *T*<sub>opt</sub>, and *T*<sub>max</sub> identified at different growth temperatures and CO<sub>2</sub> levels were similar at 14.9, 30.1, and 45.6 °C, respectively for peanut pollen. The corresponding values for sorghum pollen were 17.2, 29.4, and 41.7 °C. In conclusion, pollen longevity and pollen germination percentage was decreased by growth at elevated temperature, and pollen developed at elevated temperature and/or elevated CO<sub>2</sub> did not have greater temperature tolerance.

Published by Elsevier B.V.

## 1. Introduction

In recent years, crops across the world have experienced more variable and extreme weather events, including episodes of high temperatures during crop growing seasons. These weather events will become more aggravated because of global climate change associated with increases in concentration of greenhouse gases, deforestation, and consumption of fossil fuels (IPCC, 2007). It is estimated that by the end of this century, carbon dioxide (CO<sub>2</sub>) concentration will be in the range from 540 to 970 μmol CO<sub>2</sub> mol<sup>-1</sup> (IPCC, 2007). Increases in CO<sub>2</sub> and other greenhouse gasses will be associated with increases in global surface temperatures in the range of 1.4–5.8 °C (Schneider, 2001; IPCC, 2007). These climate changes will have a significant effect on crop production.

Seed set percentage which determines seed numbers is one of the most important components of crop yield. Seed set pri-

marily depends on the functionality of male and female gametes (pollen and ovule, respectively), which are highly sensitive to environmental factors. Environmental conditions during growth and development of reproductive organs and during and after anthesis can influence performance of gametes and seed set (Stephenson et al., 1994; Saini, 1997; Prasad et al., 2003, 2006). In general, pollen is more sensitive to environmental stress relative to the ovule. Pollen performance can be hindered by the duration for which pollen is viable and can germinate after anther dehiscence (pollen longevity) and its ability to reach and fertilize the ovule. Pollen longevity is defined as the duration for which pollen grains has the ability to germinate after dehiscence from anther. Pollen performance influences transmission of genes from one generation to the next. Thus, understanding the factors affecting performance of gametes is important (Stephenson et al., 1994).

The duration for which pollen is viable and can germinate after anther dehiscence under natural conditions is crucial for successful pollination and fertilization. Similarly, response of pollen grains to temperature is also important to understanding the physiological basis of decreased seed set under stress environments. Some studies have documented the influence of instantaneous temperature,

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humidity, and solar radiation on pollen longevity (Luna et al., 2001; Aylor, 2004). Cardinal temperatures ( $T_{\min}$ ,  $T_{\text{opt}}$ , and  $T_{\max}$ ) for pollen germination for some cultivars of crops such as peanut (*Arachis hypogaea* L. Kakani et al., 2002), common bean (*Phaseolus vulgaris* L.; Farlow et al., 1979), tomato (*Lycopersicon esculentum* Mill; Weaver and Timm, 1989) and cotton (*Gossypium hirsutum* L. and *Gossypium barbadense* L.; Burke et al., 2004) were reported at a particular growth temperature. A few studies have investigated the influence of growth conditions such as mineral nutrition (Lau and Stephenson, 1993; Poulton et al., 2002), temperature, and  $\text{CO}_2$  (Aloni et al., 2001; Prasad et al., 2002, 2003, 2006) on pollen performance, the influence of growth temperature and/or  $\text{CO}_2$  on pollen longevity or cardinal temperatures for pollen germination has received far less attention.

It has been established that processes such as photosynthesis, stomata conductance, seed number, seed size and composition of seeds, and enzyme activities in leaves and seeds are influenced by growth temperature and  $\text{CO}_2$  concentration (Reddy and Hodges, 2000; Thomas et al., 2003; Prasad et al., 2003, 2004, 2009). Studies have shown that photosynthesis and photosynthetic capacity acclimates under elevated growth temperatures and  $\text{CO}_2$  conditions (Vu et al., 1997; Bunce, 2000). However, acclimation responses of pollen grains to season-long higher growth temperatures are not well understood.

The present research was conducted on peanut and grain sorghum (*Sorghum bicolor* L. Moench.) due to their importance in semi-arid regions. The productivity of these two crops is limited by occurrences of environmental stresses during reproductive stages of crop development. The most sensitive stages to high temperature stress occur just before flowering and during flowering in peanut (Prasad et al., 1999a) and sorghum (Prasad et al., 2008). These two stages coincide with microsporogenesis and anthesis, respectively. High temperature stress during microsporogenesis causes poor pollen viability, fewer numbers of pollen grains, resulting in lower seed set. Similarly, high temperature stress during flowering causes poor anther dehiscence, poor pollen germination, slower pollen tube growth and hampers fertilization, resulting in lower seed set. Analyses of weather data suggests that both peanut (Prasad et al., 2003) and sorghum (Prasad et al., 2006) crops in the semi-arid regions are already being grown at optimum or above optimum temperature for their yield. Thus, any further increases in mean temperature or occurrences of short episodes of high temperatures during reproductive stages of crop development will decrease yields. Understanding physiological and biochemical basis of sensitivity of pollen to high temperature stress and exploring differences among crop species and cultivars within species can help determine new avenues for genetic improvement for stress tolerance.

Objectives of the present study were to (a) determine pollen longevity of grain sorghum under different growth temperatures and/or  $\text{CO}_2$  conditions, (b) determine whether temperature response of sorghum and peanut (representative of monocot  $\text{C}_4$  and dicot  $\text{C}_3$  crops of tropical region) in terms of cardinal temperatures,  $T_{\min}$  (minimum temperature at which pollen germinates),  $T_{\text{opt}}$  (optimum temperature at which pollen germination in maximum) and  $T_{\max}$  (maximum temperature beyond which pollen does not germinate) for pollen germination was influenced by temperature and/or  $\text{CO}_2$  conditions during the formation and development of pollen grains, and (c) compare pollen longevity and cardinal temperatures (i.e.  $T_{\min}$ ,  $T_{\text{opt}}$  and  $T_{\max}$ ) of two crops species (peanut and sorghum). The hypothesis tested was that growth at elevated temperature and/or  $\text{CO}_2$  does not improve tolerance of pollen performance in terms of pollen germination, pollen longevity, or cardinal temperatures for pollen germination.

## 2. Materials and methods

This research was conducted in controlled environment facilities of the University of Florida and United States Department of Agriculture at the Plant and Soil Science Field Teaching Laboratory in Gainesville, FL (latitude 29.64°, longitude 82.34°, and altitude 30 m). Details of the controlled environments and quality of environmental controls, experimental conditions, and plant husbandry from these experiments are available elsewhere (peanut, Prasad et al., 2003; and sorghum, Prasad et al., 2006), along with growth and yield data. A brief summary of growth conditions and plant husbandry is described in the following section.

### 2.1. Experimental conditions

Peanut and grain sorghum plants were grown in outdoor, sunlit Soil–Plant–Atmosphere Research (SPAR) growth chambers during 2002 and 2003, respectively. These growth chambers have unique computer controlled programs, equipment and structure to control air temperature, dew-point temperature and  $\text{CO}_2$  at pre-determined set points. Each chamber is airtight and has an upper aluminum frame measuring 1 m wide, 2 m long and 1.5 m high covered with a polyethylene terephthalate “six light” film (Taiyo Kogyo Co., Tokyo, Japan) walls which enclose the crop canopy. The bottom rooting-chamber (aluminum lysimeter) has the same cross-section as the upper frame and is 0.6 m deep. All chambers contain the same natural topsoil of Kendrick sand (loamy, siliceous, Arenic Paleudult) obtained from a nearby field (90.7% sand, 5.6% silt and 3.7% clay).

Carbon dioxide was controlled and maintained at  $350 \mu\text{mol mol}^{-1}$  in all eight chambers from sowing to appearance of first leaf; thereafter,  $\text{CO}_2$  concentration in half (four) of the chambers was increased to  $700 \mu\text{mol mol}^{-1}$ . From sowing to full emergence (7 days after sowing, DAS), the air temperature was set at 36/26 °C (daytime maximum/nighttime minimum) in all chambers; thereafter, four temperature treatments of 32/22, 36/26, 40/30, and 44/34 °C were randomly allocated to eight chambers in paired sets (one each at 350 and  $700 \mu\text{mol mol}^{-1}$ ). The  $\text{CO}_2$  and temperature control continued until final harvest at maturity. Temperature was controlled in a sinusoidal wave function and data on all environmental variables were measured at 20 min interval through the experiment.

Quality of the temperature and  $\text{CO}_2$  control and chamber performance is presented elsewhere (sorghum, Prasad et al., 2006; peanut, Prasad et al., 2003). In brief, the chamber performance was tested from the measured data on growth and dry matter production of crops in each chamber when controlled at similar environments (either temperature or  $\text{CO}_2$ ) in two different experiments (Prasad et al., 2006). Overall, the data clearly demonstrated that all chambers performed similarly and produce similar results in terms of plant development, growth and dry matter production when grown under similar environmental and crop management conditions (Supplemental Table 1). In addition the quality of the environmental controls in all chambers was similar when set at similar air temperature and/or  $\text{CO}_2$  levels (Prasad et al., 2006). Together, these data provide assurance on uniformity of the chambers (no chamber effects), chamber-conditions and reliability of data collected from experiments in SPAR growth chambers without true replications.

The set point temperature regimes for current study were chosen based on optimal temperature for growth and development of each crop. Our previous studies on peanut has shown that exposure to daytime temperatures  $>34^\circ\text{C}$  significantly decreased pollen viability, pollen production and resulted in decreased seed set and fewer number of seeds (Prasad et al., 1999b, 2001). Similarly, studies on grain sorghum have shown that daytime temperature  $>33^\circ\text{C}$

decreased seed filling duration and resulted in smaller seed size and seed yield (Prasad et al., 2006). Mean daily optimum temperature for reproductive growth of peanut and sorghum is about 28 °C.

## 2.2. Plant husbandry

Uniform seeds of Georgia Green (Virginia runner botanical-type peanut cultivar) and Dekalb 28E (semi-dwarf, photoperiod-insensitive sorghum cultivar) were treated with fungicide (Captan, 45% at rate of 200 g per 100 kg of seed) as a precautionary measure against seed-borne diseases. Seeds were sown by hand on 15 July 2002 (peanut) and 11 August 2003 (sorghum). There were two, 2-m-long rows running east to west. Plants were irrigated by overhead sprinkler irrigation from sowing to 10 days after emergence until a good root system was established. Thereafter, plants were fully dependent on subsurface irrigation provided by a constant water table at about 45 cm beneath the soil surface maintained by an external float-valve device for each chamber. There was a good capillary flow through the soil, which kept the soil near field capacity throughout the growing season. Plants were thinned soon after emergence, and a uniform population of 20 plants per square meter (40 per chamber) was maintained.

## 2.3. *In vitro* pollen germination medium

The pollen germination medium for peanut consisted of 100 mg  $\text{H}_3\text{BO}_3$ , 250 mg  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 200 mg  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 100 mg  $\text{KNO}_3$ , and 100 mg sucrose dissolved in 1 L of deionized water (Prasad et al., 2001, 2003). The pollen germination medium for sorghum consisted of 150 mg  $\text{H}_3\text{BO}_3$ , 500 mg  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 200 mg  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 100 mg  $\text{KNO}_3$ , and 150 mg sucrose dissolved in 1 L of deionized water (Prasad et al., 2006). The crop species had different pollen germination medium, as the medium for optimized for each crop to obtain maximum pollen germination percentage. In both mediums,  $20 \text{ g L}^{-1}$  of agar were added and slowly heated on a hotplate until agar was completely dissolved. Thereafter, the germinating medium was poured on the required number of glass slides and allowed to cool for 15–20 min so that agar solidified. Each glass slide layered with germinating medium was kept in an empty Petri dish lined with moistened filter paper to provide a humid atmosphere and incubated at 28 °C for 30 min before pollen grains were placed on them for measuring *in vitro* pollen germination percentage.

## 2.4. Pollen longevity

To test pollen longevity (duration for which pollen grains had the ability to germinate after anther dehiscence), flowers/florets were tagged on 15 different plants at anthesis and pollen grains were collected from those tagged floret/flowers at 30-min to 1-h intervals until 7 h after anthesis (i.e. from 0700 to 1400 h) and tested for pollen germination by *in vitro* pollen tests. The position of node (middle of the main stem) or panicle branch (middle of the panicle) on which florets were tagged and collected was similar across all treatments. All timings are given in Eastern Standard Time (EST). In the peanut experiment, pollen longevity was tested in 32/22 °C at ambient  $\text{CO}_2$ . In the sorghum experiment, pollen longevity was tested at two temperatures (32/22 and 36/26 °C) at both ambient and elevated  $\text{CO}_2$  treatments. For sorghum, there was no panicle emergence at growth temperatures of 40/30 and 44/34 °C, thus no pollen could be collected. Pollen was collected from about 5 florets from 5 different tagged plants, mixed thoroughly on the glass slide, and evenly spread on the slide containing germinating medium. The process of pollen collection and extraction was repeated three times (replications) from different set of plants. Slides were then kept at room temperature (26 °C) for 30–45 min in

the dark, and pollen germination in each slide was measured. A total of about 100–200 grains were counted on each slide. Percentage of pollen germination was estimated by counting the total number of pollen grains and number of germinated pollen grains in a random microscopic field on each microscopic slide using compound light microscope (Nikon Instruments Inc., Melville, NY). Pollen was considered germinated if the length of the pollen tube was greater than the diameter of the pollen grain.

## 2.5. Temperature response

To determine the response of *in vitro* pollen germination to temperature, pollen grains from different growth temperature and  $\text{CO}_2$  treatments were collected at the time of anthesis (0630–0730 h, EST). Petri dishes and microscopic slides were incubated at different temperatures ranging from 12 to 48 °C at 4 °C in the incubators (Ecotherm Chilled Incubators Model 70i, Research Product International, Mt. Prospect, IL, USA) under dark conditions for 30 min. Thereafter, pollen grains were spread on germinating medium and kept at respective temperatures in different incubators. Procedures for pollen sampling and estimation of pollen germination were similar to those described previously. The position of nodes or panicle branch from which flowers/florets were collected was similar across all treatments. There were three slides for each measurement (repetitions) and there were three replications for each treatment.

## 2.6. Cure fitting and data analyses

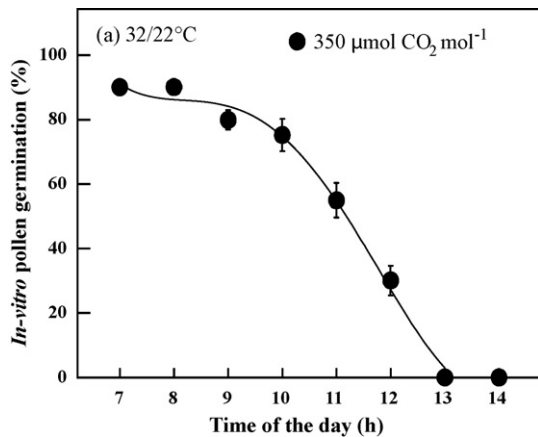
General linear model (GLM) and nonlinear model procedures in SAS (SAS Institute Inc., Cary, NC, USA) were used to identify treatment differences and temperature responses (Kakani et al., 2002, 2005; Salem et al., 2007). Various regression equations (models) for the response of pollen germination were compared  $R^2$  and root mean square deviation (RMSD) were determined. The best fit was determined by the model which provided the highest  $R^2$  and lowest RMSD. The response of peanut pollen to temperature was best described by a bilinear model, whereas, response of sorghum pollen to temperature was best described by a quadratic model. Regression procedures for respective models were used estimated cardinal temperatures ( $T_{\min}$ ,  $T_{\text{opt}}$  and  $T_{\max}$ ) using previously published procedures (Kakani et al., 2002, 2005; Salem et al., 2007). There were three replications for each treatment. Mean and standard errors were used to determine differences between pollen longevity over time and pollen germination at different temperatures. The influence of growth temperature,  $\text{CO}_2$ , and interaction of temperature and  $\text{CO}_2$  on pollen longevity and temperature response of pollen was analyzed by comparison of regression techniques in SAS.

# 3. Results

## 3.1. Pollen longevity

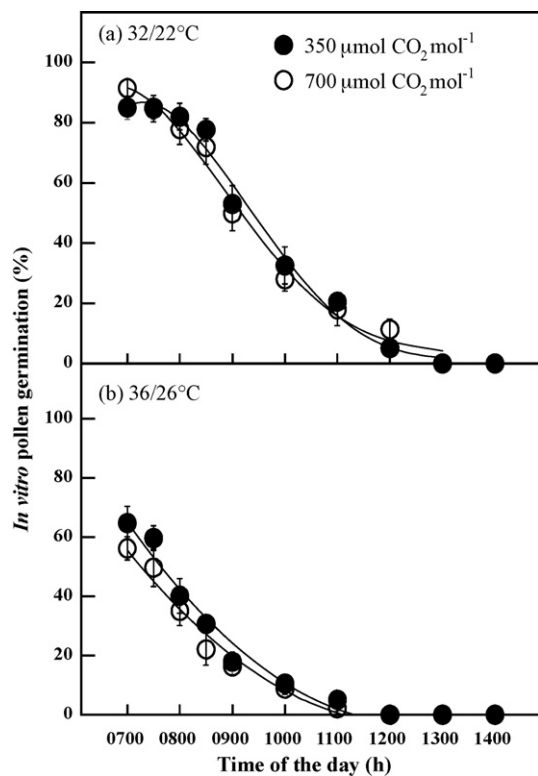
Pollen longevity as measured by *in vitro* pollen germination at different times after anther dehiscence varied between peanut and sorghum at 32/22 °C (Figs. 1 and 2). Pollen germination in peanut decreased from about 90% at anthesis to 75% 3 h later; thereafter, pollen decreased linearly and lost complete function 6 h after anthesis at growth temperature regime of 32/22 °C at ambient ( $350 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$ .

Pollen longevity in sorghum was significantly influenced by temperature but not by  $\text{CO}_2$  or interaction between temperature and  $\text{CO}_2$  (Table 1). At growth temperature of 32/22 °C, pollen germination remained the same for about 1 h after anthesis and started to decline exponentially and lost complete function 5 h after anthesis



**Fig. 1.** Longevity of pollen as determined by *in vitro* pollen germination of peanut at growth temperature of 32/22 °C at ambient CO<sub>2</sub> (●, 350 μmol CO<sub>2</sub> mol<sup>-1</sup>). Each datum represents mean and standard error of three replications. Timings are given in Eastern Standard Time (EST). Regression line:  $Y = (((0.85X - 11.40)X + 165.2)X - 1039.9)X + 2501.1$ ,  $r^2 = 0.99$ ,  $P < 0.01$ .

at both ambient and elevated CO<sub>2</sub> (Fig. 2a). At growth temperature regime of 36/26 °C, germination started to decline exponentially soon after anthesis and lost its ability to germinate after 4 h (Fig. 2b). The rate of decline in pollen germination and time taken to reach zero pollen germination was similar at ambient or elevated CO<sub>2</sub>. Comparison of pollen germination percentages at different timings (0700 through 1000 h) showed that pollen germination percent-



**Fig. 2.** Longevity of pollen as determined by *in vitro* pollen germination of sorghum at growth temperature of (a) 32/22 °C and (b) 36/26 °C under ambient (●, 350 μmol CO<sub>2</sub> mol<sup>-1</sup>) and elevated CO<sub>2</sub> (○, 700 μmol CO<sub>2</sub> mol<sup>-1</sup>). Each datum represents mean and standard error of three replications. Timings are given in Eastern Standard Time (EST). Regression lines: (a)  $Y = (((-0.206X + 9.23)X - 150.5)X + 1042.3)X - 2508.9$ ,  $r^2 = 0.99$ ,  $P < 0.01$ , at ambient; and  $Y = (((-0.175 + 7.57)X - 118.8)X + 784.9)X - 1758.5$ ,  $r^2 = 0.99$ ,  $P < 0.01$ , at elevated CO<sub>2</sub>; (b)  $Y = (2.32X - 57.47)X + 353.9$ ,  $r^2 = 0.98$ ,  $P < 0.01$ , at ambient; and  $Y = (2.07X - 50.99)X + 311.1$ ,  $r^2 = 0.98$ ,  $P < 0.01$ , at elevated CO<sub>2</sub>.

**Table 1**

Significance of temperature, CO<sub>2</sub> and interaction between temperature and CO<sub>2</sub> on various traits of peanut and sorghum pollen.

Trait	Temperature	CO <sub>2</sub>	Temperature × CO <sub>2</sub>
Peanut			
Pollen longevity	—	—	—
Pollen germination (%)	***	NS	NS
$T_{min}$ (minimum temperature)	NS	NS	NS
$T_{opt}$ (optimum temperature)	NS	NS	NS
$T_{max}$ (maximum temperature)	NS	NS	NS
Sorghum			
Pollen longevity	***	NS	NS
Pollen germination (%)	***	*	*
$T_{min}$ (minimum temperature)	NS	NS	NS
$T_{opt}$ (optimum temperature)	NS	NS	NS
$T_{max}$ (maximum temperature)	NS	NS	NS

NS = non-significant at  $P$  levels >0.05 – not measured.

\* Significant at  $P$  levels of 0.05.

\*\*\* Significant at  $P$  levels of 0.001.

ages at growth temperature of 36/26 °C (Fig. 2b) were significantly ( $P < 0.001$ ) lower than those at 32/22 °C (Fig. 2a).

### 3.2. Temperature response of pollen

Temperature response of peanut pollen was best described by a bilinear model (nonlinear broken-stick model, i.e. with linear increase from  $T_{min}$  to  $T_{opt}$  and linear decrease from  $T_{opt}$  to  $T_{max}$ ). There were no significant effects of growth temperature, CO<sub>2</sub>, or interaction between temperature and CO<sub>2</sub> on temperature response or cardinal temperatures for pollen germination (Table 1). Cardinal temperatures ( $T_{min}$ ,  $T_{opt}$ , and  $T_{max}$ ) as estimated by the bilinear model in peanut were 15.0, 30.8, and 45.9 °C at growth temperature of 32/22 °C (Fig. 3a). Corresponding values were 14.5, 29.8, and 46.0 °C at growth temperature of 36/26 °C; 14.0, 29.0, and 46.0 °C at growth temperature of 40/30 °C; and 16.0, 30.8, and 45.0 °C at growth temperature of 44/34 °C (Fig. 3b–d, respectively). The slope of increase or decrease in pollen germination was similar at 32/22 °C and 36/26 °C but greater than at 40/30 or 44/34 °C.

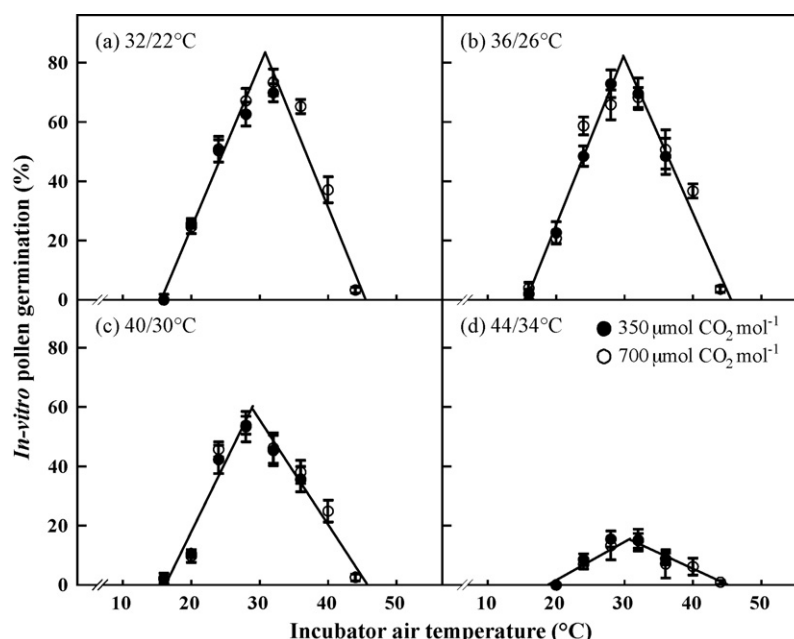
Temperature response of sorghum pollen was best described by a quadratic function (Fig. 4). There were no significant effects of temperature, CO<sub>2</sub>, or interaction between temperature and CO<sub>2</sub> on temperature response of pollen (Table 1). The cardinal temperatures identified by the quadratic model were 17.0, 29.3, and 41.6 °C at growth temperature of 32/22 °C under both ambient and elevated CO<sub>2</sub> (Fig. 4a). Corresponding values were 17.3, 29.5, and 41.7 °C at growth temperature of 36/26 °C under both ambient and elevated CO<sub>2</sub> (Fig. 4b).

There were significant effects of growth temperature on absolute values of pollen germination percentage, which decreased with increasing temperature in both crop species. For example, in peanut at close optimum temperature (30 °C), pollen germination was 79%, 82%, 65%, and 14% at growth temperatures of 32/22, 36/26, 40/30, and 44/34 °C, respectively (Fig. 3). Similarly, pollen germination at close optimum temperature (30 °C) in sorghum decreased from about 68% to 40% as growth temperature increased from 32/22 to 36/26 °C under ambient CO<sub>2</sub>. There were no significant effects of CO<sub>2</sub>, or interaction between temperature and CO<sub>2</sub> on absolute values of pollen germination percentage across all temperatures in peanut. In sorghum, however, at growth temperature of 36/26 °C, pollen germination at elevated CO<sub>2</sub> was lower than that at ambient CO<sub>2</sub>, particularly at close to optimum temperatures (Fig. 4b).

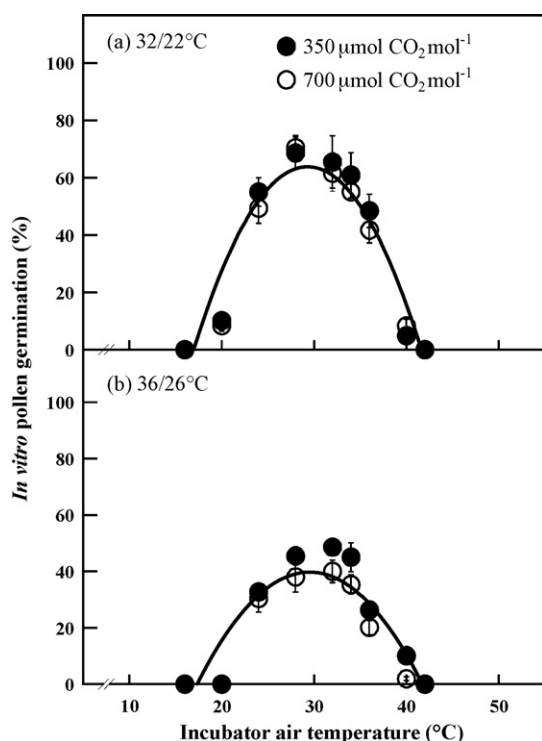
## 4. Discussion

This study on two crop species, peanut and sorghum, suggests that growth temperature significantly affects ability of pollen to





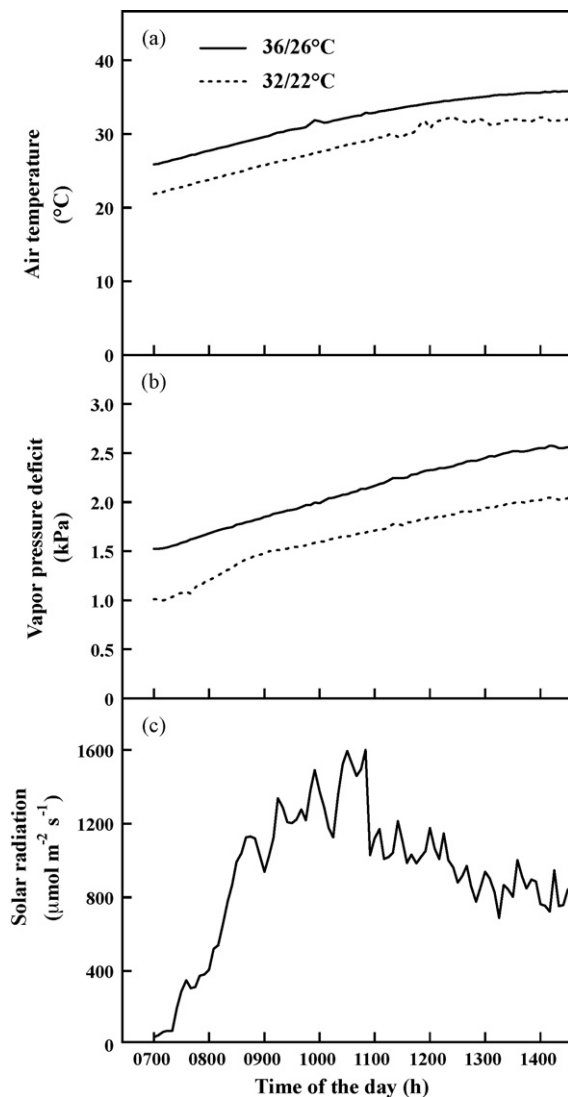
**Fig. 3.** Temperature response of peanut pollen formed and developed at growth temperatures of (a) 32/22°C, (b) 36/26°C, (c) 40/30°C, and (d) 44/34°C at ambient (●, 350  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) and elevated  $\text{CO}_2$  (○, 700  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) as determined by *in vitro* pollen germination at different incubator temperatures. Each datum represents mean and standard error of three replications. Single regression was drawn for data at ambient and elevated  $\text{CO}_2$ . Regression lines: (a)  $Y = 5.5X - 85.87$ ,  $r^2 = 0.98$  (when  $X < 30.8$ ) and  $Y = -5.6X + 258.3$ ,  $r^2 = 0.96$  (when  $X \geq 30.8$ ); (b)  $Y = 5.77X - 90.23$ ,  $r^2 = 0.98$  (when  $X < 29.8$ ) and  $Y = -5.24X + 238.5$ ,  $r^2 = 0.97$  (when  $X \geq 29.8$ ); (c)  $Y = 4.71X - 76.14$ ,  $r^2 = 0.93$  (when  $X < 29.0$ ) and  $Y = -3.53X + 161.7$ ,  $r^2 = 0.96$  (when  $X \geq 29.0$ ); and (d)  $Y = 1.29X - 24.34$ ,  $r^2 = 0.91$  (when  $X < 30.5$ ) and  $Y = -1.06X + 17.93$ ,  $r^2 = 0.95$  (when  $X \geq 30.5$ ). Regressions significant at  $P < 0.01$ .



**Fig. 4.** Temperature response of sorghum pollen formed and developed at growth temperatures of (a) 32/22°C, and (b) 36/26°C, at ambient (●, 350  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) and elevated  $\text{CO}_2$  (○, 700  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) as determined by *in vitro* pollen germination at different incubator temperatures. Each datum represents mean and standard error of three replications. Single regression was drawn for data at ambient and elevated  $\text{CO}_2$ . Regression lines: (a)  $Y = (-0.419X + 24.56)X - 296.2$ ,  $r^2 = 0.91$ ,  $P < 0.01$ ; (b)  $Y = (-0.267X + 15.78)X - 193.28$ ,  $r^2 = 0.85$ ,  $P < 0.05$ .

germinate but does not improve pollen longevity or cardinal temperatures ( $T_{\min}$ ,  $T_{\text{opt}}$ , and  $T_{\max}$ ) of pollen grains. Sorghum pollen had relatively shorter longevity compared with peanut pollen (Figs. 1 and 2). Similarly, decreases in pollen germination at growth temperatures of 36/26°C compared with 32/22°C were greater in sorghum (26%) than in peanut (5%) when measured at 32°C (Figs. 3b and 4b). Crop species have different pollen longevity. For example, rice (*Oryza sativa* L.) pollen is viable for 5–10 min (Pan and Dong, 1994), corn (*Zea mays* L.) pollen for 1 day, and pennisetum (*Pennisetum alopecuroides* Spreng.) pollen for 3–5 days (Li and Chen, 1998; Fu et al., 2001) when stored at 35°C. A detailed review (Dafni and Firmage, 2000) of pollen longevity of various plants including crop and wild grass species suggest that there is wide variation in pollen longevity and it can range from as short as 5 min in rice (Koga et al., 1971; Pan and Dong, 1994) and wheat (*Triticum aestivum* L.; Athwal and Kimber, 1970) to 6 days in *Brassica campestris* L. (Katiyar and Gupta, 1985) and 8 days in *Colopogon tuberosus* L. (Proctor, 1998). Cultivars within species can also differ in pollen longevity. For example, pollen germination of peanut cv. ICGS-11 grown during rainy season of 1995 in Junagadh (Gujarat, India) decreased from 85% to 24% after 8 h, whereas in cv. GG-11, pollen viability decreased from about 88% to 56% in the same time period (Chandran and Rajagopal, 1997).

Pollen longevity in sorghum was shorter (4 h) at growth temperature of 36/26°C than at 32/22°C (6 h). Decreased longevity of sorghum pollen at high temperatures could be a result of disruption of carbohydrate accumulation in pollen grains and/or changed the ultrastructure of pollen grain at high temperature (Jain et al., 2007). In addition, the quicker loss of moisture from pollen (Buitink et al., 1996; Luna et al., 2001) due to high temperatures and high vapor pressure deficit. Air temperature and vapor pressure deficit at growth temperature treatments of 32/22 and 36/26°C are shown in Fig. 5. Sorghum pollen longevity in our study (hybrid DK 28E) was much longer (4–6 h) than that observed by Lansac et al. (1994), where pollen of four different



**Fig. 5.** Environmental conditions: (a) air temperature, (b) vapor pressure deficit, and (c) solar radiation in different growth temperature treatments at the time of testing for pollen longevity in sorghum during 2003. Because these environmental conditions were similar in both ambient and elevated CO<sub>2</sub> treatments, only data from ambient CO<sub>2</sub> is shown for clarity. Timings are given in Eastern Standard Time (EST).

genotypes was viable for only 30 min even when stored at 25 °C and 50% relative humidity. This differential response may be due to cultivar and/or growth conditions of the crop or pollen germination medium (liquid or semisolid agar medium). Experiments conducted by collecting pollen from different growth temperatures at the same time and determining pollen longevity by storing at a set point temperature would provide useful information on pollen longevity and best conditions for pollen storage for crop breeding. This will be of practical importance due to limited time available for plant breeders for pollination while developing cultivars.

Cardinal temperatures ( $T_{\min}$ ,  $T_{\text{opt}}$ , and  $T_{\max}$  = 14.89, 30.1, and 45.8 °C, respectively) identified in this study for pollen germination of peanut cv. Georgia Green were similar to those previously identified for other peanut cultivars (Kakani et al., 2002). Furthermore, the  $T_{\text{opt}}$  and  $T_{\max}$  temperatures for pollen germination identified here were similar to those for seed set (i.e. 32 and 44 °C, respectively; Prasad et al., 2003). Our data on sorghum showed the cardinal temperatures for pollen viability were 17.2, 29.4, and

41.7 °C, respectively. This is in contrast to observations of Tunistra and Wedel (2000), which showed no influence of temperature in the range of 20–40 °C. This differential response may be related to cultivars, which are known to differ in crops such as peanut (Kakani et al., 2002), soybean (*Glycine max* L. Merr; Salem et al., 2007), tomato (Weaver and Timm, 1989) and *Capsicum* sp. (Reddy and Kakani, 2007).

The shape of temperature response of pollen germination was not influenced by high temperature and/or elevated CO<sub>2</sub> in peanut or sorghum (Figs. 3 and 4). There were no differences in terms of cardinal temperatures ( $T_{\min}$ ,  $T_{\text{opt}}$  or  $T_{\max}$ ), which were similar under different growth temperatures and CO<sub>2</sub>. Furthermore, the fact that pollen grains developed at a higher growth temperature did not have improved pollen germination when germinated at optimum or cooler temperature (Figs. 3 and 4) suggests there was no acclimation of pollen grains to high temperature. Although several researchers have shown acclimation of vegetative processes such as photosynthesis under elevated temperature, CO<sub>2</sub>, and light conditions (Sage et al., 1989; Vu et al., 1997; Bunce, 2000; Niinemets and Valladares, 2004), acclimation of reproductive processes such as pollen longevity or pollen germination to environmental stresses are not well understood.

Development of floral organs occurs separate from vegetative organs, thus the response to high temperature stress is different. Most vegetative tissue exhibits heat shock response, but germinating pollen does not exhibit the heat shock protein and results in rapid loss of viability upon exposure to high temperature stress (Hopf et al., 1992). Relative to heat shock response in vegetative tissues, response of pollen was weak, and also, subsets of heat shock proteins were present in low amounts in pollen grain (Fravo et al., 1991; Mascarenhas and Crone, 1996). In recent years, more pollen-expressed genes have been identified, and studies are showing large differences between genes express in pollen and other plant tissues (Costa-Nunes and Grossniklaus, 2003). Our recent study on sorghum suggest that high temperature stress caused starch deficiency in the developing pollen leading to decreased pollen germination leading to lower seed set (Jain et al., 2007). It was determined that impairment of cell wall invertase-mediated sucrose hydrolysis and subsequent lack of sucrose biosynthesis may be the most upstream molecular dysfunctions leading to altered carbohydrate metabolism and start deficiency under high temperature conditions (Jain et al., 2007). Studies identifying such mechanisms and showing comprehensive analyses of molecular events underlying heat shock response in pollen are limited (Frank et al., 2009). Thus, further studies on composition, proteins, enzymes, and their transcripts during different stages of pollen development at different growth temperatures are necessary to identify processes of acclimation and heat shock response in pollen grains. Similarly, comparing effects of sudden and gradual temperature increases will prove useful for developing a better understanding of pollen acclimation to high temperatures.

We conclude that both pollen longevity and pollen germination percentage were decreased by growth at elevated temperature and that pollen grains developed at higher growth temperatures did not have greater  $T_{\min}$ ,  $T_{\text{opt}}$  or  $T_{\max}$ . There was no effect of growth temperature and/or CO<sub>2</sub> on shape of the temperature response curve or cardinal temperatures for pollen germination in both crop species. Future research should focus on carbohydrates contents, heat shock proteins, enzyme activities, and ultrastructure of pollen grains of crops species under different growth temperatures. It will also be important to investigate whether cultivars within species vary in pollen longevity in ambient or stressed environments. Such information will prove useful for helping breeders identify tolerant cultivars and/or understand the physiological basis of high temperature tolerance and developing options for crop management.

## Acknowledgements

We thank Larry Pitts and Wayne Wynn for excellent engineering support and Dr. Jean Thomas for technical support. Financial support from University for Florida, United States Department of Agriculture – Agricultural Research Service, and the Peanut Collaborative Research Support Program (CRSP) and International Sorghum and Millet CRSP (INTSORMIL) of United States Aid for International Development is acknowledged. This is contribution no. 09-183-J from the Kansas Agricultural Experiment Station.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envexpbot.2010.08.004.

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